Environmental Health Perspectives
Vol. 60, pp. 411-421, 1985

Interaction of Alkylmercuric Compounds with Sodium Selenite II. Metabolism of Methylmercuric Chloride Administered Alone and in Combination with Sodium Selenite in Rats

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Repeated doses of sodium selenite (Se) were administered to rats receiving repeated (IV or PO) doses of 0.25 or 2.5 mg Hg/kg methylmercuric chloride ($Me2^{203}Hg$).

Se (0.5 mg/kg) was observed to alter the distribution of $Me^{203}Hg$ among tissues as well as among subcellular fractions of kidneys and liver. An excess of selenium resulted in a twofold decrease in the mercury content of kidneys and a similar increase in the mercury content of brain.

Introduction

The problems of toxicity, uptake, distribution and excretion of methylmercury in humans and experimental animals have been dealt with by many authors and presented in numerous reports (*1–5*) monographs (*6–15*), and current communications (*16–20*). However, most reports have concentrated on specific aspects of these problems. Complex studies considering dose-dependent retention, excretion, distribution (organ and subcellular) and binding of methylmercuric chloride to cell components depending on the dose and route of administration of methylmercury are lacking.

Although clinical symptoms of toxicity of methymercuric compounds are well known (*2*), trials of treatment or amelioration of its toxic effects are still in the experimental stage.

It has been established that interaction of selenium with inorganic mercury results in a decreased uptake of mercury at the site of its administration and decreases its excretion with urine and feces. The concentrations of mercury in the liver and blood are significantly enhanced, but simultaneously the mercury content of the kidney is significantly reduced (*21–24*). These changes are most pronounced when mercury and selenium are administered at at least equimolar doses (*21–25*). The sequence of administration of both these elements is also important. The effect of selenium on the distribution of mercury is smaller when the selenium is administered after mercury than in the case of simultaneous exposure to both these metals (*26,27*).

The protective action of sodium selenite against the nephrotoxic effect of inorganic mercury (*13,28–34*) and the beneficial action of selenium in methylmercury poisoning has been described by many authors (*33,35– 47*). Sodium selenite administration results in delayed occurrence of symptoms of neurological and histological disturbances and in enhanced life expectancy of exposed animals (*40,43,48*).

Results of studies on the selenium–methylmercury interaction are sometimes contradictory. Selenium has been reported to increase whole-body retention of methylmercury (*35*), increase levels of methylmercury in brain, liver, blood, and spleen while reducing the mercury content at kidneys. The effect on brain, noted by many investigators (*41,49–51*), is of special interest, since no symptoms of intoxication were observed in animals receiving sodium selenite simultaneously with methylmercury, even though the concentration of methylmercury in their brain exceeded critical values (*13,15*). In other studies simultaneous administration of selenium was reported to result in significant changes in the distribution of methylmercury in the body (*34,41,45*). There are also reports stating that if selenium affects the methylmercury concentration in the blood at all, it re-

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sults in a decrease rather than an increase (*52*) and that this effect is attributable to a selenium-induced decrease in the affinity of red blood cells for methylmercury (*52*).

These discrepancies prompted the present study of the effect of selenium on excretion, whole-body retention, and organ and subcellular distribution and binding of selenium to proteins of the soluble fraction of the liver and kidneys. An effort has been made to relate the results to dose and route of methylmercuric chloride administration in rats.

Materials and Methods

Female Wistar rats, body weight 150–200 g, fed standard LSM diet and allowed to drink tap water *ad libitum* were used in this study. The animals were divided into eight groups. Data on the group size, compounds administered, routes of administration and doses applied are given in Table 1.

The animals were exposed to the metals for 2 weeks. During exposure rats were kept in metabolic cages, one animal per cage. Depending on the body weight, the animals received solutions of appropriate compounds in volumes of 0.38–0.62 cm^2 intragastrically and 0.15–0.20 cm intraveneously per single dose. Selenium (Se) was given intragastrically as water solution of sodium selenite (Na_2SeO_3; POCh, Gliwice, Poland) every day at single doses of 0.5 mg Se/kg.

Methylmercuric chloride (MeHg, K&K Laboratories Inc., Plainview, NY, USA), labeled with $^{203}Hg(CH_3{}^{203}HgCl$, Radiochemical Centre, Amsterdam, Bucks, England) of specific activity 51.8 μCi/mg was given every other day intragastrically in 0.1% sodium carbonate (Na_2CO_3; POCh, Gliwice, Poland) or intraveneously in 0.9% sodium chloride (NaCl, POCh, Gliwice, Poland).

Total excretion of mercury in feces and urine was monitored for each animal daily. At 24 hr after the last administration of methylmercury, rats were sacrificed under ether narcosis by heart puncture, and individual organs and tissues were isolated.

Mercury was determined directly in urine, feces, and tissues after grinding or homogenization and suspending in starch, by γ-counting in an USB-2 scintillation counter with a NaI/Tl crystal. The counting time was 100 sec; the accuracy of counting was ± 10%.

Perfused liver and kidneys were fractionated by the method described elsewhere (*53*) following the procedures of Shibko et al. (*54*) and Lucier and McDaniel (*55*). In kidney and liver homogenates and in successive supernatants ^{203}Hg was estimated by a radiochemical method and protein concentration was measured (*56*).

Separation of mercury-binding proteins in the soluble fraction of the kidneys and liver was performed by gel filtration on Sephadex G-75(2.5 60 cm). The columns were eluted with formate buffer, pH 8.0; 3-cm^3 fractions were collected at a rate of 10 cm/hr. The columns were calibrated with: Dextran Blue (molecular weight of 2,000,000; Pharmacia, Sweden) cytochrome C (molecular weight of 12,700; Serva, West Germany) and potassium chromate (molecular weight of 194; POCh, Gliwice, Poland). Mercury was assayed radiochemically in column eluates and protein concentration was monitored by measurements of absorbance at 280 and 250 nm in a VSU-2P spectrophotometer. All determinations were made separately for each animal.

Results

During the exposure, rats exposed to the lower dose of methyl mercuric chloride (0.25 mg Hg/kg) excreted about 62 μg Hg after intragastric administration (group I, Fig. 1), and about 50 μg Hg after intravenous injection (group II, Fig. 2) which amounted to about 20% and 15%, respectively, of the cumulative dose of this metal.

Rats exposed to the higher dose of methylmercury (2.5 mg Hg/kg) excreted about 250 μg ^{203}Hg, i.e., about 8% of the total metal administered, irrespective of the route of administration, i.e., intragastric (group III) or intravenous (group IV) (Figs. 3 and 4 and Table 2).

Sodium selenite administered both at an equimolar dose with respect to mercury (groups IIIa and IVa) (Figs. 3 and 4) and at a tenfold excess (groups Ia and IIa) (Figs. 1 and 2) had little effect on the amount of mercury excreted, irrespective of the route of administration of methylmercury. A tendency for decreased mercury excretion was observed only with the tenfold selenium excess. This effect was observed both in the daily and cumulative excretion of ^{203}Hg in urine and feces.

The whole-body retention of ^{203}Hg was about 78% after a dose of 0.25 mg Hg/kg and about 70% after a dose of 2.5 mg Hg/kg (Table 2), irrespective of the route of administration of methylmercury.

Table 1. Characteristics of experimental animal groups.

Group	Number of animals	Compounds administered	Dose of mercury, mg Hg/kg	Route of administration of Hg	Hg:Se molar ratio
I	6	MeHg	0.25	Intragastric	—
Ia	5	MeHg + Se	0.25	"	1:10
II	4	MeHg	0.25	Intravenous	—
IIa	5	MeHg + Se	0.25	"	1:10
III	5	MeHg	2.5	Intragastric	—
IIIa	5	MeHg + Se	2.5	"	1:1
IV	5	MeHg	2.5	Intravenous	—
IVa	5	MeHg + Se	2.5	"	1:1

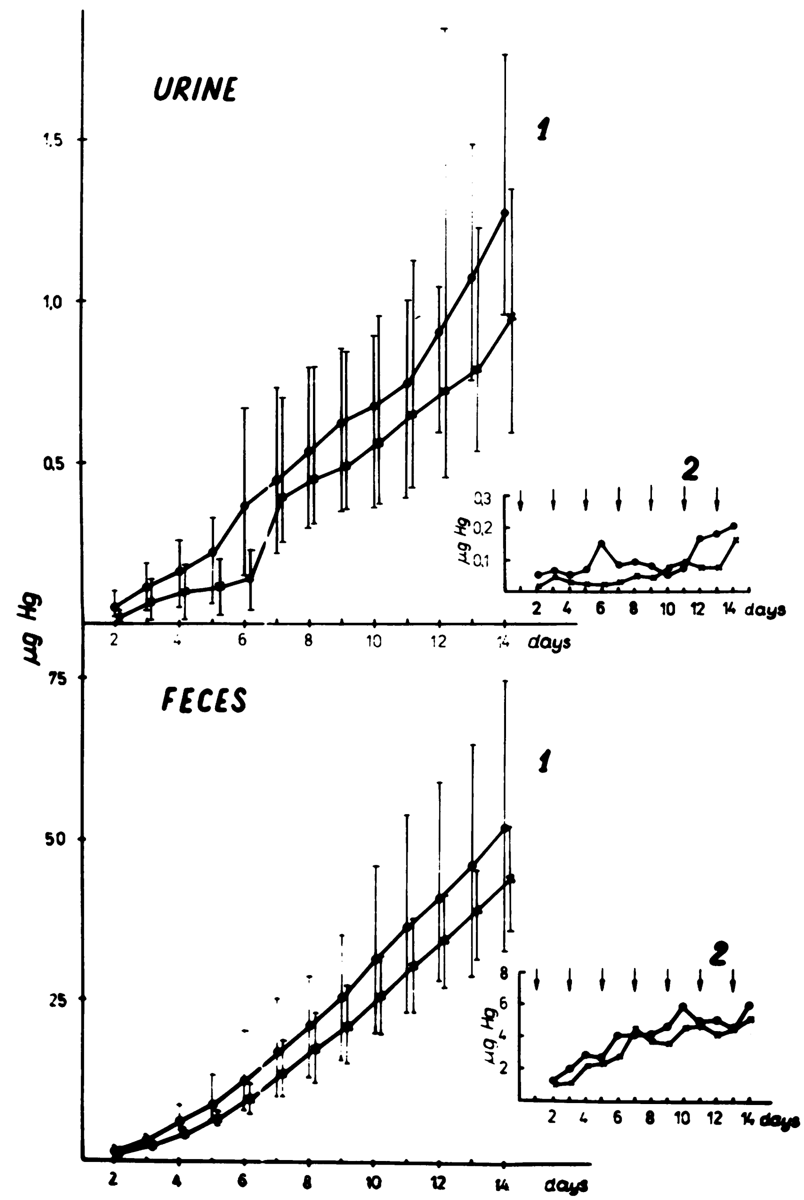

FIGURE 1. Cumulative (1) and daily (2) excretion of $Me^{203}Hg$ in urine and feces during 2-week intragastric exposure of rats to $Me^{203}HgCl$ ± Se: (○) group I, 0.25 mg/Hg/kg; (×) group Ia, 0.25 mg Hg/kg +Se. Bars represent range from five animals.

The concentrations of mercury in tissues of rats exposed intragastrically (group I) or intraveneously (group II) to the lower dose of methylmercury (0.25 mg Hg/kg) are shown in Table 3. For both routes of exposure, the lowest concentration (1–3 µg Hg/g tissue) were found in the brain, lungs, heart, liver, intestines, muscles, bones, and skin. Mercury concentrations in the spleen and blood and kidneys were 7 and 10 µg Hg/g tissue, respectively.

A tenfold higher dose of methylmercury (2.5 mg Hg/kg) supplied either intragastrically (group III) or intraveneously (group IV) resulted in a proportional tenfold increase in the concentration of ^{203}Hg in respective tissues (Table 4).

Sodium selenite supplied at a tenfold excess with respect to mercury given both intragastrically (group Ia) and intraveneously (group IIa) elevated the concentration of ^{203}Hg in the brain about twofold and decreased it in the blood and kidneys (Table 3).

An equimolar dose of selenium with respect to mercury had the greatest effect on the concentration of ^{203}Hg in the brain of rats, increasing it as in the case of selenium excess (Table 4).

Due to the similarity of results for both routes of administration of methylmercury, concentrations of ^{203}Hg in organelles of the liver and kidneys expressed per milligram protein are shown jointly in Tables 5 and 6 without indicating the type of exposure.

In animals exposed to the low dose of methylmercury (0.25 mg Hg/kg) the concentrations of ^{203}Hg in subcel-

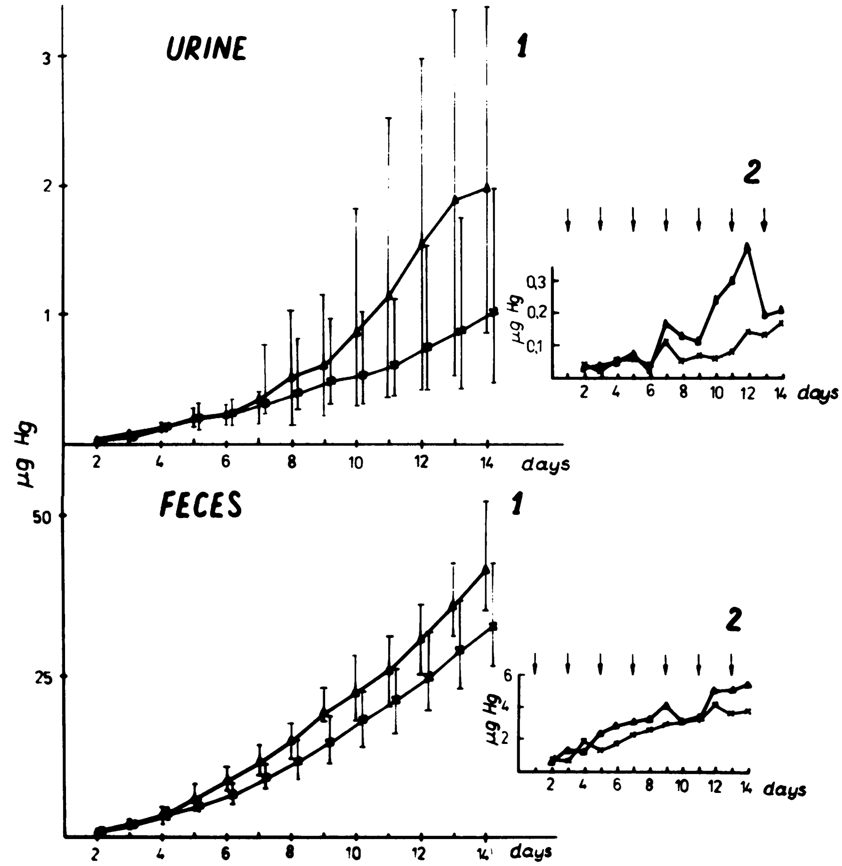

FIGURE 2. Cumulative (1) and daily (2) excretion of $Me^{203}Hg$ in urine and feces during 2-week intravenous exposure of rats to $Me^{203}Hg$ ± Se: (▲) group II, 0.25 mg Hg/kg; (×) group IIa, 0.25 mg Hg/kg + Se. Bars represent range from five animals.

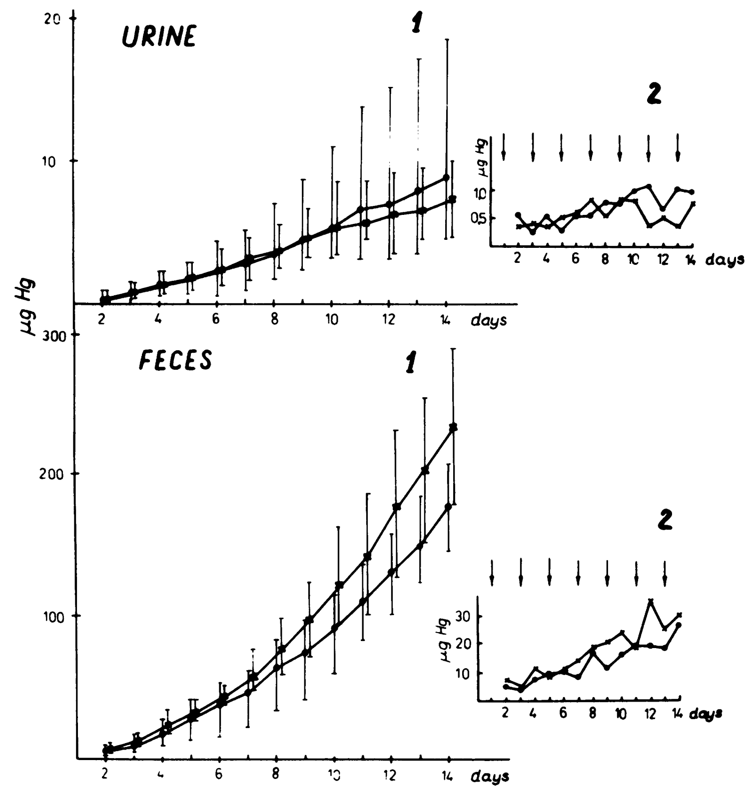

FIGURE 3. Cumulative (1) and daily (2) excretion of $Me^{203}Hg$ in urine and feces during 2-week intragastric exposure of rats to $Me^{203}HgCl$ ± Se: (●) group III, 2.5 mg Hg/kg; (×) group IIIa, 2.5 mg Hg/kg + Se. Bars represent range from five animals.

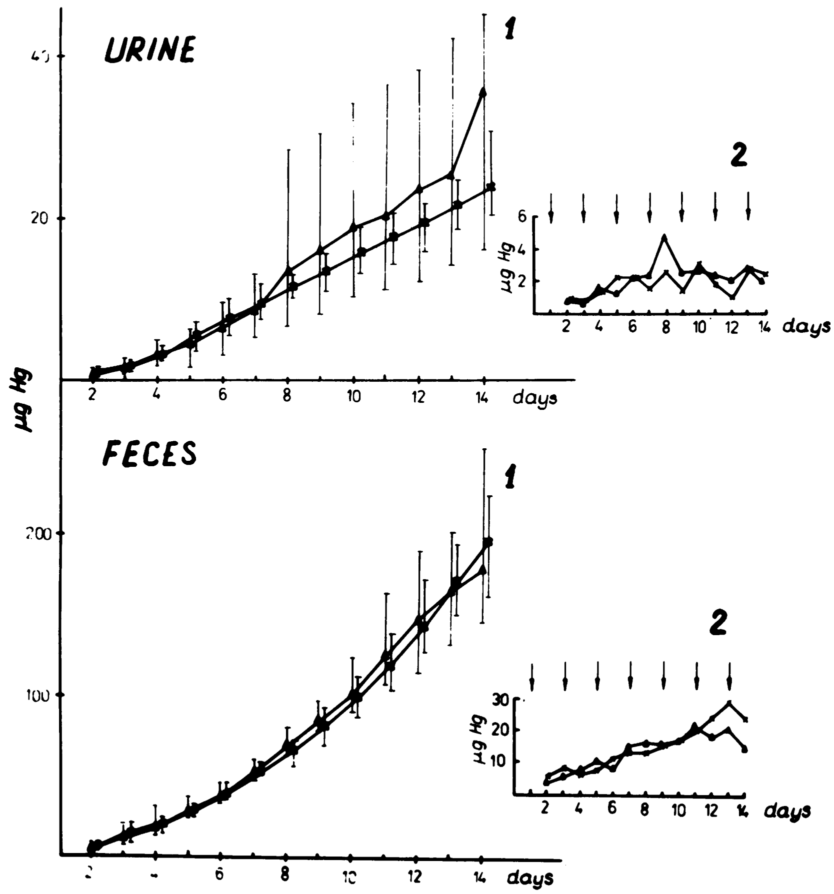

FIGURE 4. Cumulative (1) and daily (2) excretion of $Me^{203}Hg$ in urine and feces during 2-week intravenous exposure of rats to $Me^{203}HgCl$ ± Se: (▲) group IV, 2.5 mg Hg/kg (×) group IVa, 2.5 mg Hg/kg + Se. Bars represent range from five animals.

Table 2. Whole body retention (mean and range) and whole body to whole blood content of Me^{203} Hg ratio after 2 week exposure of rats to methylmercuric chloride with or without sodium selenite.

Group	Treatment	Whole-body retention, %[a]	Whole body / Whole blood
I	0.25 mg Hg/kg, PO	77.3 72.7–82.8	5.5
II	0.25 mg Hg/kg, IV	78.4 74.1–81.2	4.4
III	2.5 mg Hg/kg, DO	69.1 60.2–82.0	4.5
IV	2.5 mg Hg/kg, IV	70.4 66.3–75.8	4.2
Ia	0.25 mg Hg/kg, PO + Se	79.0 74.8–85.2	6.8
IIa	0.25 mg Hg/kg, IV + se	86.6 68.2–120.5	6.2
IIIa	2.5 mg Hg/kg, PO + Se	81.0 71.9–92.3	4.2
IVa	2.5 mg Hg/kg, IV + Se	77.2 63.9–102.3	5.3

[a]Mean and range.

lular fractions of the liver ranged from 0.005 μg Hg/mg protein in the mitochondrial fraction to 0.031 μg Hg/mg protein in the soluble fraction.

After exposure to the higher level of methylmercury (2.5 mg Hg/kg), the highest concentrations of ^{203}Hg were found in the light lysosomal and microsomal fractions.

In the soluble fraction of the liver, retaining this metal with the highest efficiency irrespective of the routeof administration and dose of methylmercury, ^{203}Hg was bound almost completely by high molecular weight proteins (Fig. 5) and the amount of metal bound to proteins depended only on the concentration of ^{203}Hg in the total soluble fraction. Sodium selenite did not affect the binding pattern of mercury to proteins of the soluble fraction of the liver.

The excess of selenium practically did not alter the level of mercury (per mg protein) in subcellular fractions of the liver. The only exception was the soluble fraction in which the concentration of mercury decreased (Table 5).

An equimolar dose of selenium induced a considerable dimunition of the level of mercury in the light lysosomal fraction and a simultaneous increase of its level in the remaining fractions. The highest elevation took place in the microsomal fraction (Table 5).

In the kidneys of rats exposed to the lower dose of methylmercury (0.25 mg Hg/kg) the concentration of ^{203}Hg referred to the protein content (Table 6) was the highest in the microsomal, soluble and membrane fractions.

After application of the tenfold higher dose of meth-

Table 3. Concentration of methylmercury in rat tissues after 2-week intragastric exposure to $Me^{203}HgCl$ with or without sodium selenite (mean ± SD).

Tissue	$Me^{203}Hg$, μg/g tissue[a] Group I, 0.25 mg Hg/kg	Group Ia, 0.25 mg Hg/kg + Se	Group III, 2.5 mg Hg/kg	Group IIIa, 2.5 mg Hg/kg + Se
Liver	1.62 ± 0.44	1.64 ± 0.26	16.39 ± 3.92	20.50 ± 5.63
Kidneys	8.02 ± 1.54	4.41 ± 1.60†	79.12 ± 7.98	67.69 ± 10.46*
Spleen	3.32 ± 1.43	3.66 ± 0.72	45.70 ± 11.67	39.64 ± 6.48
Heart	2.19 ± 0.36	2.14 ± 0.59	16.91 ± 1.54	17.44 ± 3.68
Brain	0.83 ± 0.29	1.59 ± 0.23†	8.38 ± 1.87	15.59 ± 2.11†
Lung	2.30 ± 0.42	2.01 ± 0.28	18.23 ± 1.94	19.37 ± 3.88
Stomach	0.70 ± 0.18	0.65 ± 0.42	8.91 ± 5.14	12.29 ± 6.63
Intestines	1.04 ± 0.34	0.90 ± 0.20	8.67 ± 2.75	8.38 ± 2.41
Tail	0.77 ± 0.06	0.85 ± 0.33	6.95 ± 3.23	9.30 ± 1.27
Skin	1.10 ± 0.48	1.15 ± 0.27	14.04 ± 5.10	12.66 ± 3.73
Muscle and bone	1.04 ± 0.08	1.16 ± 0.06*	7.93 ± 1.58	8.71 ± 1.53
Blood	6.93 ± 0.47	5.57 ± 0.29†	80.17 ± 10.90	76.18 ± 10.13
Plasma	0.09 ± 0.03	0.08 ± 0.02	0.96 ± 0.28	0.86 ± 0.14

[a]Mean ± SD.
*Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.05$.
†Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.01$.

Table 4. Concentration of methylmercury in rat tissues after 2-week intravenous exposure to $Me^{203}HgCl$ with or without sodium selenite (mean ± SD).

Tissue	$Me^{203}Hg$, μg/g tissue[a] Group II, 0.25 mg Hg/kg	Group IIa, 0.25 mg Hg/kg + Se	Group IV, 2.5 mg Hg/kg	Group IVa, 2.5 mg Hg/kg + Se
Liver	1.65 ± 0.04	1.77 ± 0.26	15.13 ± 2.09	23.39 ± 6.83*
Kidneys	10.67 ± 1.75	4.56 ± 1.10†	68.52 ± 10.71	59.24 ± 10.13
Spleen	4.58 ± 0.84	4.05 ± 1.64	30.98 ± 12.38	41.53 ± 13.56
Heart	1.99 ± 0.29	1.90 ± 0.46	14.78 ± 4.08	23.32 ± 6.19*
Brain	0.77 ± 0.14	1.56 ± 0.28†	6.15 ± 2.20	15.94 ± 4.31†
Lung	1.91 ± 0.13	1.98 ± 0.50	13.84 ± 3.18	20.79 ± 5.07†
Stomach	0.53 ± 0.19	0.55 ± 0.25	5.45 ± 1.48	6.70 ± 2.00
Intestines	0.85 ± 0.13	0.85 ± 0.28	8.11 ± 2.56	8.61 ± 2.22
Tail	1.85 ± 0.58	2.19 ± 0.53	22.82 ± 10.15	25.39 ± 10.42
Skin	0.78 ± 0.06	0.92 ± 0.67	12.54 ± 2.23	9.12 ± 4.61
Muscle and bone	1.04 ± 0.02	1.29 ± 0.46	7.28 ± 0.60	9.55 ± 2.85
Blood	7.27 ± 0.88	5.56 ± 1.11*	69.32 ± 9.19	63.38 ± 16.41
Plasma	0.17 ± 0.06	0.11 ± 0.04	0.64 ± 0.13	0.58 ± 0.19

[a]Mean ± SD.
*Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.05$.
†Significantly different.

Table 5. Methylmercury in subcellular fractions of rat liver after 2-week exposure to $Me^{203}HgCl$ with or without sodium selenite (mean ± SD).

Subcellular fractions	$Me^{203}Hg$, μg/g tissue[a] Groups I and II, 0.25 mg Hg/kg	Groups Ia and IIa, 0.25 mg Hg/kg + Se	Groups III and IV, 2.5 mg Hg/kg	Groups IIIa and IVa, 2.5 mg Hg/kg + Se
H	0.010 ± 0.002	0.010 ± 0.001	0.096 ± 0.022	0.162 ± 0.059*
M_s	0.006 ± 0.003	0.007 ± 0.002	0.084 ± 0.038	0.138 ± 0.076
N	0.005 ± 0.002	0.009 ± 0.005	0.074 ± 0.022	0.087 ± 0.020
M	0.005 ± 0.002	0.007 ± 0.004	0.062 ± 0.020	0.169 ± 0.136*
L_h	0.014 ± 0.008	0.010 ± 0.004	0.069 ± 0.034	0.123 ± 0.123
L_l	0.012 ± 0.002	0.011 ± 0.005	0.590 ± 0.525	0.207 ± 0.084
P	0.020 ± 0.007	0.019 ± 0.014	0.264 ± 0.189	0.675 ± 0.421*
S	0.025 ± 0.007	0.016 ± 0.002	0.164 ± 0.039	0.259 ± 0.105*

[a]Mean ± SD.
*Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.05$.

Table 6. Methylmercury in subcellular fractions of rat kidneys after 2-week exposure to $Me^{203}HgCl$ with or without sodium selenite (mean ± SD).

Subcellular fractions	$Me^{203}Hg$, μg/g tissue[a]			
	Groups I and II, 0.25 mg Hg/kg	Groups Ia and IIa, 0.25 mg Hg/kg + Se	Groups III and IV, 2.5 mg Hg/kg	Groups IIIa and IVa, 2.5 mg Hg/kg + Se
H	0.068 ± 0.012	0.027 ± 0.007[†]	0.499 ± 0.054	0.402 ± 0.038[†]
M_s	0.096 ± 0.052	0.027 ± 0.008[†]	0.387 ± 0.138	0.375 ± 0.106
N	0.046 ± 0.018	0.021 ± 0.012[†]	0.481 ± 0.100	0.267 ± 0.067[†]
M	0.057 ± 0.032	0.022 ± 0.013[*]	0.438 ± 0.263	0.317 ± 0.034
L_h	0.048 ± 0.020	0.050 ± 0.028[*]	0.451 ± 0.230	0.543 ± 0.152
L_l	0.059 ± 0.020	0.027 ± 0.013[†]	0.707 ± 0.183	0.237 ± 0.042[†]
P	0.122 ± 0.020	0.060 ± 0.036[†]	0.684 ± 0.305	0.804 ± 0.278
S	0.096 ± 0.033	0.039 ± 0.007[†]	0.703 ± 0.135	0.573 ± 0.064

[a]Mean ± SD.
[*]Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.05$.
[†]Significantly different from group of rats receiving the same dose of MeHg without selenium, $p < 0.01$.

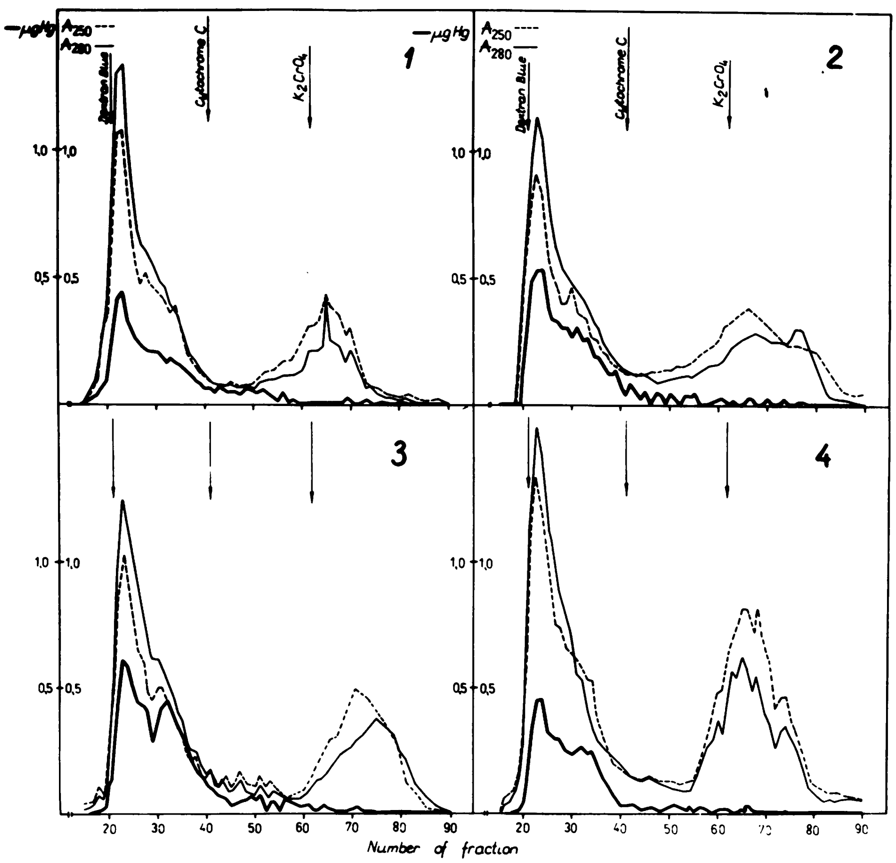

FIGURE 5. Separations of soluble fraction of rat liver after 2-week intragastric and intravenous exposure to $Me^{203}HgCl$ ± Se: (1) groups I and II (0.25 mg Hg/kg); (2) groups Ia and IIa (0.25 mg Hg/kg + Se); (3) groups III and IV (2.5 mg Hg/kg); (4) groups IIIa and IVa (2.5 mg Hg/kg + Se). Sephadex G-75 column eluted with buffer as described in Methods section: (——) A_{280}; (– –) A_{250}, (—)μg ^{203}Hg. Arrows indicate the position of Dextran Blue, cytochrome C, and K_2CrO_4.

ylmercury the highest concentrations of mercury were found in the soluble, light lysosomal and microsomal fractions.

In the kidneys of rats given 2.5 mg Hg/kg the soluble fraction had the highest contribution to the accumulation of ^{203}Hg, as in the case of the lower dose (Table 6).

The excess of selenium with respect to mercury (Groups Ia and IIa) which decreased the concentration of mercury in the kidneys (Tables 3 and 4) resulted in a simultaneous diminution of the concentration of ^{203}Hg (as referred to the protein) in all subcellular fractions. The highest decrease took place in the membranes. The only exception was the heavy lysosomal fraction in which selenium induced an increase in the concentration of mercury. On the other hand, an equimolar dose of selenium does not elevate the concentrations of ^{203}Hg in the subcellular fractions of kidneys (Table 6).

The binding of ^{203}Hg by proteins of the subcellular fractions of rat kidneys was dependent only on the dose of methylmercury and the presence of sodium selenite and was independent of the route of administration of methylmercuric chloride. The pattern of binding of ^{203}Hg to proteins of the soluble fraction of the kidneys as a function of the dose of methylmercuric chloride and the presence of selenium is shown in Figure 6.

In rats exposed to the low dose of methylmercuric chloride (0.25 mg Hg/kg, groups I and II) mercury was bound by proteins of the soluble fraction of the kidneys eluted in three distinct peaks (Fig. 6). High molecular weight proteins bound 35.6 and 40.7% of mercury, depending on the molecular weight; protein of molecular weight of about 10,000 (probably metallothioneinlike proteins) linked about 20.4% of ^{203}Hg accumulated in this fraction in the kidneys.

In the case of the higher dose (2.5 mg Hg/kg) of methylmercuric chloride (groups III and IV) mercury was bound in the form of two peaks to high molecular weight proteins and to low molecular weight proteins (metallothionein), with 34.3, 44.5, and 18.8% of the total metal contained in this fraction, respectively (Fig. 6).

Sodium selenite administered at a tenfold excess with respect to mercury (groups Ia and IIa) brought about a considerable decrease in the amount of mercury bound to high molecular weight proteins and practically totally displaced mercury from metallothioneinlike proteins (Fig. 6). High molecular weight proteins of the soluble fraction of kidneys of rats of these groups bound, depending on the molecular weight, about 37.2 and 55.8% of ^{203}Hg retained in the fraction.

Discussion

Results presented in this paper may allow determination of the possibility of forecasting the methylmercury concentration in rat tissues on the basis of determination of its concentration in blood, and the effect of the presence of sodium selenite on such estimations.

In the study, two different routes of administration and two significantly different doses of methylmercury (0.25 and 2.5 mg Hg/kg) were employed. This permitted us to obtain different mercury:selenium ratios at a constant dose of selenium (Table 1).

The studies performed indicate that, irrespective of the dose and route of administration, the same percent of the cumulative dose of methylmercury was excreted in urine and feces (Figs. 1–4) and probably with expired air (*1,14*). As a result, the percent whole-body retention of methylmercury after repeated exposure was similar in all cases and amounted to about 70% of the cumulative

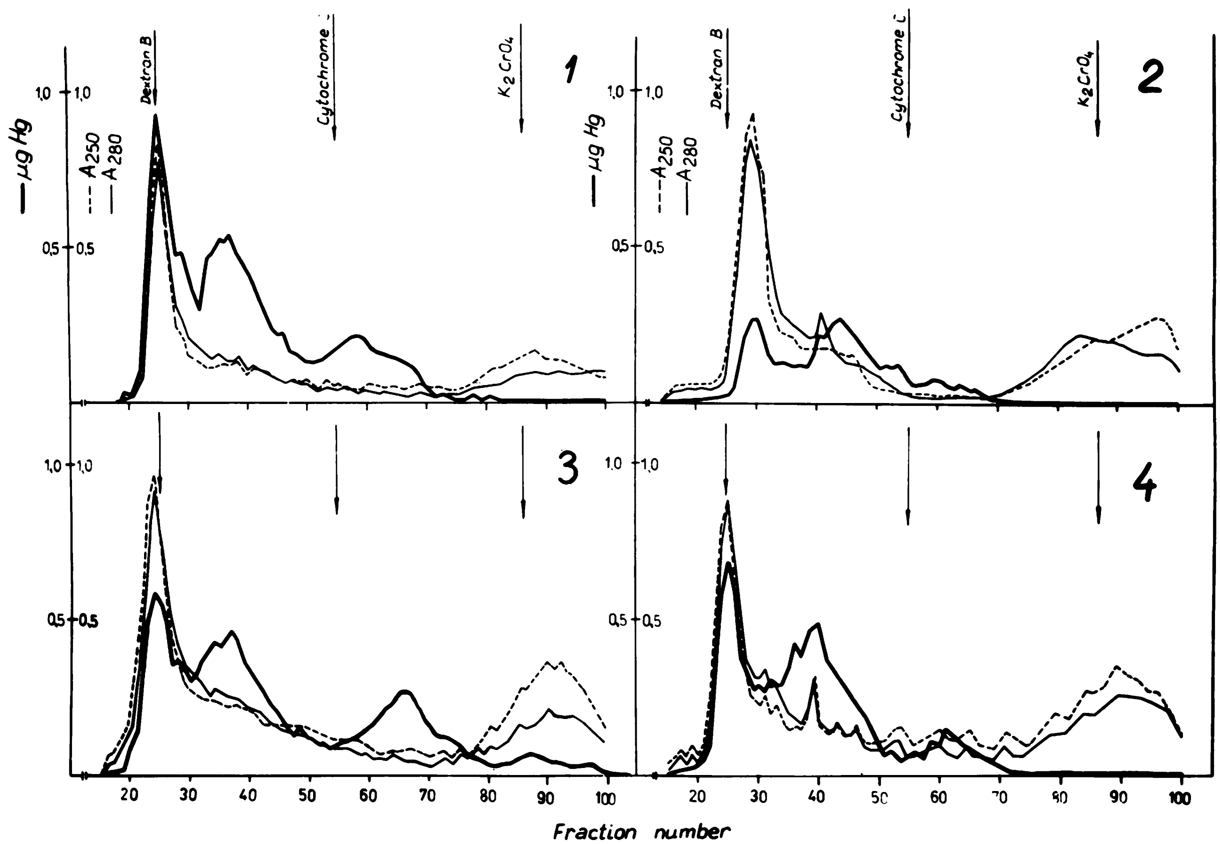

FIGURE 6. Separations of soluble fraction of rat kidneys after 2-week intragastric and intravenous exposure to Me^{203}HgCl ± Se: (1) groups I and II (0.25 mg Hg/kg); (2) groups Ia and IIa (0.25 mg Hg/kg + Se); (3) groups III and IV (2.5 mg Hg/kg); (4) groups IIIa and IVa (2.5 mg Hg/kg + Se). Sephadex G-75 column eluted with buffer as described in Methods section: (——) A_{280}; (– –) A_{250}; (▬)μg ^{203}Hg. Arrows indicate the position of Dextran Blue, cytochrome c and K_2CrO_4.

Table 7. Tissue Hg to blood Hg concentration ratio in rats after administration of methylmercuric compounds.

Treatment	Dose pattern	Brain/Blood	Liver/Blood	Kidney/Blood	Reference
7 × 0.25 mg Hg/kg, PO	Repeated	0.12	0.23	1.17	Table 3
7 × 0.25 mg Hg/kg, IV	Repeated	0.11	0.23	1.46	Table 4
7 × 2.5 mg Hg/kg, PO	Repeated	0.09	0.18	1.01	Table 3
7 × 2.5 mg Hg/kg, IV	Repeated	0.09	0.22	1.01	Table 4
10 × 1.0 mg Hg/kg, SC	Repeated	0.14	0.29	0.86	(*57*)
9 × 13 μg Hg/rat, PO	Repeated	0.07	0.28	1.12	(*19*)
9 × 1 μg Hg/rat, PO	Repeated	0.08	0.29	1.25	(*19*)
0.84 mg/kg or 3.34 mg/kg, PO	Repeated	0.06	0.27	1.07	(*58*)
100 μg Hg/rat, IV	Single	0.17	0.27	0.71	(*59*)
34 mg Hg/kg, PO	Single	0.07	0.26	0.53	(*60*)
116 μg Hg/rat, IV	Single	0.18	0.23	1.14	(*61*)

dose. Upon termination of the exposure, the ratio of the whole-body content of methylmercury to its content in the blood was also almost independent of the dose and route of administration and close to 5 (Table 2).

Our finding that in repeated exposure to methylmercury its concentrations in individual tissues increased approximately proportionally to the administered dose (Tables 3 and 4) seems noteworthy. Owing to this phenomenon, values of the methylmercury concentration ratios tissue:blood were very similar if not identical for both routes of administration and both doses, especially in the case of such vital organs as brain, liver, and kidneys (Table 7). This observation may allow in the future for an estimation of methylmercury concentration in the tissues on the basis of its concentration in the blood, especially when using similar conditions of exposure for different purposes. Very similar values of these ratios can be derived from data of other authors (*19,57,58*) who also employed repeated exposure and, like us, determined methylmercury concentration in tissues and in blood soon (usually 24 hr) after termination of the exposure (Table 7). Our calculations show that values of those ratios are similar also after single administration of methylmercury (Table 7) if methylmercury concentrations in blood and tissues in short times after exposure are considered (*59–61*).

The presence of selenium, though increasing the whole-body retention of methylmercury only slightly (Table 2) changed its levels in individual tissues significantly, especially in the kidneys and brain, irrespective of the dose and route of administration of the latter (Tables 3 and 4). This is reflected by significantly altered numerical values of the tissue: blood methylmercury concentration ratios (Table 8). Therefore, an estimate of the tissues concentrations of methylmercury in the rat on the basis of its blood concentration may be charged with a large error in the presence of selenium. This refers especially to the brain and kidneys, where the increase and decrease, respectively, of this ratio is dependent on the molar concentrations of mercury and selenium. It results from the available data that an increase in the methylmercury concentration in the rat brain takes place not only with an excess of selenium (Table 3) or equimolar concentrations of both elements (Table 4) but also

Table 8. Tissue Hg to blood Hg concentration ratio in rats after simultaneous methylmercury and selenium administration.

Treatment	Brain/Blood	Liver/Blood	Kidney/Blood	Reference
0.25 mg Hg/kg, PO + Se	0.29	0.29	0.78	Table 3
0.25 mg Hg/kg, IV + Se	0.28	0.32	0.82	Table 4
2.5 mg Hg/kg, PO + Se	0.20	0.27	0.89	Table 3
2.5 mg Hg/kg, IV + Se	0.26	0.37	0.95	Table 4

when the molar dose of selenium was lower than the molar dose of methylmercury (expressed as metalic mercury). This effect was observed for both single (*50,51*) and repeated administration of methylmercury. However the mechanism involved remains unknown.

On the other hand, selenium affects the level of methylmercury in rat kidneys significantly, and in this case a clearcut diminution of the methylmercury concentration is attained only when selenium excess with respect to mercury is employed (Table 3). This effect is observed in all subcellular fractions of this organ; in the soluble fraction, the decrease includes the amount of methylmercury bound to both high molecular weight and low molecular weight protein fractions (Fig. 6). As a result, the kidneys:blood methylmercury concentration ratio is decreased, especially for selenium excess (Table 8).

Numerous studies indicate that the interaction effect of selenium and inorganic mercury is different and is characterized by a clear-cut translocation of mercury from low molecular to high molecular weight kidney proteins (*24,26,27,64*) already at equimolar concentrations of mercury and selenium (*27*). This phenomenon is accompanied by about a fivefold diminution of mercury concentration in the kidneys and inhibition of metallothionein biosynthesis (*26,27,64–66*). Simultaneously a distinct, about fourfold increase of the level of this metal is observed, especially in the mitochondrial and nuclear fractions (*26*) and concentration of mercury in the blood increases considerably (*25,27,67*). Such effects are not observed in methylmercury–selenium interaction. In this case no increase but rather a decrease in the methylmercury concentration is found in the blood, regardless of whether selenium was administered at an equimolar dose (Table 4) or in slight (*65*) or considerable

(Table 3) excess. Usually it is accompanied by only a small increase of the methylmercury concentration in the liver (Tables 3 and 4) (*65*). The binding pattern of $Me^{203}Hg$ to proteins of the soluble fraction of the kidneys (Fig. 6) points to a possible participation of metallothionein-like proteins in this process (*15,68*). That is probably due to the higher efficiency of biotransformation of methylmercury to inorganic mercury in rat kidneys as compared with liver (*58,65,69,70*); this process seems to be strictly dependent on the dose of methylmercury which has been taken into account in our further studies (*71*).

This work was performed within the framework of Project MZIX, Occupational Medicine, of the Ministry of Health, Polish People's Republic.

We wish to thank Mrs. Honorata Debicka for excellent technical assitance and for her help in the preparation of the figures.

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